Clinical, Cellular, and Molecular Features of an Israeli Xeroderma Pigmentosum Family with a Frameshift Mutation in the XPC Gene: Sun Protection Prolongs Life

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An Ashkenazi Jewish Israeli family with two children affected with severe xeroderma pigmentosum was investigated. A son, XP12TA, developed skin cancer at 2 y and died at 10 y. A daughter, XP25TA, now 24 y old, was sun protected and began developing skin cancers at 10 y. Their cultured skin fibroblasts showed reductions in post-ultraviolet survival (11% of normal), unscheduled DNA synthesis (10% of normal), global genome DNA repair (15% of normal), and plasmid host cell reactivation (5% of normal). Transcription-coupled DNA repair was normal, however. Northern blot analysis revealed greatly reduced xeroderma pigmentosum complementation group C mRNA. A plasmid host cell reactivation assay assigned the cells to xeroderma pigmentosum complementation group C. Cells from both parents and an unaffected child exhibited normal post-ultraviolet-C survival and normal DNA repair. Sequencing the xeroderma pigmentosum complementation group C cDNA of XP12TA and XP25TA revealed a homozygous deletion of two bases (del AT 669–670) in exon 5 with a new termination site 10 codons downstream that is expected to encode a truncated xeroderma pigmentosum complementation group C protein. Sequence analysis of the xeroderma pigmentosum complementation group C cDNA in cells from the parents found identical heterozygous mutations: one allele carries both the exon 5 frameshift and an exon 15 polymorphism and the other allele carries neither alteration. Cells from the unaffected brother had two normal xeroderma pigmentosum complementation group C alleles. This frameshift mutation in the xeroderma pigmentosum complementation group C gene led to reduced DNA repair with multiple skin cancers and early death. Sun protection delayed the onset of skin cancer and prolonged life in a sibling with the same mutation. ***Key words: cancer genetics/DNA polymorphism/DNA repair/skin cancer/sun protection. J Invest Dermatol 115:974–980, 2000***

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease associated with extreme sensitivity to sunlight, increased freckling, and a high incidence of neoplasms in sun-exposed parts of the body in association with defective DNA repair (Kraemer and Slor, 1985; Bootsma *et al*, 1998; Kraemer, 1999a, b). Some XP patients may develop neurologic abnormalities (Kraemer *et al*, 1987). XP has been identified in all races throughout the world (Kraemer *et al*, 1987) and is more common in the middle East and Japan than in Europe and the U.S.A.

Genetic complementation identified seven XP complementation groups (XPA-G) with deficient DNA nucleotide excision repair (Bootsma *et al*, 1998). XP complementation group C (XPC) is the most prevalent form among North Americans and Europeans (Bootsma *et al*, 1998; Kraemer, 1999b) but has not been reported in Israel. In normal cells DNA repair occurs preferentially in actively transcribed genes (transcription-coupled repair, TCR) and subsequently in the remainder of the genome (global genome repair, GGR) (Bootsma *et al*, 1998). XP cells in all complementation groups have defective GGR. XPC cells uniquely have defective GGR but normal TCR.

XPC patients exhibit elevated frequency of skin cancers but rarely have neurologic abnormalities. The molecular defects responsible for the XPC clinical phenotype were analyzed in only 18 XPC patients (Legerski and Peterson, 1992; Li *et al*, 1993; Khan *et al*, 1998; Cleaver *et al*, 1999; Chavanne *et al*, 2000), and none was from Israel. Here we report the identification of a homozygous AT deletion in exon 5 of the XPC gene in two Israeli siblings with severe XP. The resulting frameshift created a new stop codon 10 codons downstream from the mutation.

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Abbreviations: CS, Cockayne syndrome; GGR, global genome repair; TCR, transcription coupled repair; UDS, unscheduled DNA synthesis; XP, xeroderma pigmentosum; XPC, xeroderma pigmentosum complementation group C.

MATERIALS AND METHODS

Cell lines and culture conditions Fibroblast cell cultures were established from skin biopsies of two XP patients (XP12TA, XP25TA), their parents (XPH26TA, XPH27TA), and a healthy brother (35TA) (**Fig 1**). XP1TA and XP3TA skin fibroblasts were established from XP patients of complementation group D and XP1BE was established from an XPC patient (Robbins *et al*, 1974). CS1TA skin fibroblasts are from a patient with Cockayne syndrome (CS). HSTA and 96TA skin fibroblasts were obtained from unaffected healthy donors. Normal control fibroblast culture C5RO was a generous gift from Dr. J. Hoeijmakers, Erasmus University, Rotterdam, The Netherlands. Normal control cells CRL1876 were obtained from the American Type Culture Collection, Rockville, MD. Cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 40 mM L-glutamine and with 15% fetal bovine serum (FBS) (Beit Haemek, Israel). In some experiments antibiotics were added (penicillin, streptomycin, mycostatin mix; Beit Haemek) and used according to the manufacturer's recommendations. For unscheduled DNA synthesis (UDS) measurements cells were cultured in MEMα/FBS20. Incubations of cell cultures were in a 37°C humidified 5% CO_2 incubator. All Israeli patients and donors signed a consent form approved by the Tel Aviv University Medical School.

Post-ultraviolet-C (UVC) cell survival Cell survival was assessed by measuring the colony-forming ability of UVC-irradiated cells (germicidal Philips UVC lamp, predominantly 254 nm). Briefly, cells were obtained from a culture 24 h after splitting and 250 cells were seeded per 60 mm plate in triplicate using F10 medium supplemented with 40 mM L-glutamine and 20% FBS (Beit Haemek). After 24 h medium was removed and cells were rinsed once with phosphate-buffered saline (PBS) pH 7 and UV irradiated at doses of 0–8 J per m^2. Complete F10 medium was added and cells were incubated for 7–10 d until colonies could be observed microscopically. Medium was removed and the colonies were stained for 30 min with 0.2% crystal violet in 50% ethanol, rinsed in water, air dried, and counted.

UDS UDS was performed as described previously (Kraemer *et al*, 1975) with the following modifications. Cells were inoculated into two-well glass tissue culture chamber slides (Nunc) (100,000 cells per well in 2 ml MEMα/FBS20 with penicillin and streptomycin), and were re-fed with MEMα/FBS1 the next day. Six days after inoculation one chamber per slide was treated with 10 J per m^2 UVC after rinsing and refilling wells with PBS. Radiolabeling was with methyl ^{3}H-thymidine (Amersham), 10 μCi per ml MEMα/FBS1, for 2 h, followed by chamber wall and gasket removal from slide, fixation, autoradiography with 50% NTB-2 emulsion (≈2 d), developing, and staining with nuclear fast red. Grain counting was performed within an approximately 6 × 6 mm area in the center of the wells, using three slides (three irradiated and three unirradiated chambers) per culture, with about 35 apparently non-S-phase nuclei per chamber. Nuclei with grain counts greater than two standard deviations above the mean were discarded as presumed unrecognized S-phase nuclei, after which the mean was recomputed and used to determine grains per nucleus.

DNA excision repair replication DNA excision repair was determined using pulse-chase ^{3}H-BrUdR labeling post UVC exposure and analyzing the isolated DNA in the heavy-light peak after ultracentrifugation in CsCl as described previously (Slor and Cleaver, 1978). Briefly, fibroblast cells in culture dishes (DMEM/15%FBS) were incubated for 24 h with 0.1 μCi per ml ^{14}C-thymidine (Nuclear Center, Negev, Israel) to prelabel cellular DNA. Medium was replaced with DMEM/15%FBS containing 10^{-6} M FUdR and 1 mM BrUdR (unlabeled). After 60 min the medium was removed, cells were UVC irradiated (or sham treated in control cultures), and medium with ^{3}H-BrUdR in place of thymidine was added. Cells were pulsed for 60 min in radioactive medium to allow DNA repair. Cellular thymidine synthesis is effectively inhibited by the presence of the FUdR. The medium was then replaced with nonradioactive medium (chase) for a further 60 min incubation. DNA was extracted and ultracentrifuged for 36 h. About 30 fractions were collected from the bottom of the tube and aliquots were precipitated with 7% cold trichloroacetic acid, filtered through GF/C filters, and counted for radioactivity. The ratio of the ^{3}H/^{14}C in the heavy/light peak (representing DNA from cells not in S-phase) was used as a measure of DNA repair.

TCR This assay measures the resumption of RNA synthesis in damaged cells as a result of DNA repair associated with transcription activity. The method is slightly modified from the original procedure described by Mayne and Lehmann (1982). Briefly, cells were incubated for 48 h in the presence of 0.01 μCi per ml ^{14}C-thymidine (Nuclear Center, Israel) to prelabel cellular DNA. Duplicate or triplicate UV-irradiated (12 J per m^2) or control unirradiated cell-culture plates were pulse-labeled for 60 min with ^{3}H-uridine (5 μCi per ml; Amersham) either immediately after UV irradiation or after 24 h of incubation, to measure RNA synthesis before and after DNA repair. DNA and RNA were precipitated in ice-cold trichloroacetic acid and collected on nitrocellulose membrane filters (Millipore); radioactivity of the ^{14}C DNA and ^{3}H RNA was determined by scintillation counting. All RNA synthesis data were normalized to DNA concentration using the ratio of ^{3}H/^{14}C. RNA synthesis in cells prior to UV damage was taken as 100%. RNA synthesis immediately after UV damage was used as the initial inhibition. RNA synthesis 24 h post UV irradiation represented the "recovery of RNA synthesis" from the initial inhibited level immediately after damage, expressing DNA repair in actively transcribed genes. Recovery of RNA synthesis is calculated from the ratio of RNA synthesis in UV-irradiated cells to RNA synthesis in unirradiated cells.

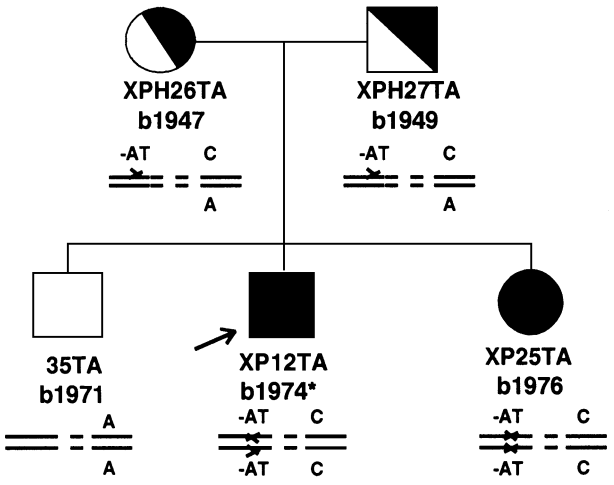


Figure 1. Pedigree of Israeli XPC family. The affected proband XP12TA (*arrow*) and his affected sister XP25TA have a homozygous mutation (del AT 669–70) in exon 5 of the XPC gene. Both parents XPH26TA and XPH27TA are clinically normal and are heterozygous for the same mutation. The clinically normal brother 35TA does not have this XPC mutation. The parental allele with the XPC mutation also carries a polymorphic marker in exon 15 2920C whereas the normal allele is 2920A.

Northern blotting Total cytoplasmic RNA was isolated from cells, separated by electrophoresis, and transferred to a positively charged nylon membrane. Northern blot hybridization was performed as described previously (Khan *et al*, 1998) using a ^{32}P-labeled XPC cDNA fragment 3.5 kb in size. Autoradiographic band intensities were measured with a laser densitometer (Molecular Dynamics, Sunnyvale, CA). After stripping off the XPC probe the same membrane was re-probed with β-actin cDNA as an internal standard.

Host cell reactivation and complementation group assignment The pCMVLuc reporter gene plasmid (a generous gift from M. Hedayati and L. Grossman, Johns Hopkins University, Baltimore, MD) was used to measure post-UV host cell reactivation. In this construct the original SV40 promoter of the luciferase gene containing plasmid pGL3 (Promega) was replaced with a CMV promoter. Host cell reactivation (Protic-Sabljic and Kraemer, 1985) was performed as described previously (Emmert *et al*, 2000). Briefly, 25 μl of CsCl-purified pCMVLuc either UV irradiated (1000 J per m^2) or unirradiated was transfected into 0.15×10^6 cells seeded onto 35 mm culture dishes using 3 μl lipofectamine (BRL Gibco) in a total volume of 1 ml for 5 h. After 48 h luciferase activity was measured with a luminometer (Monolight 2010, Analytical Luminescence Laboratory, San Diego, CA) using Luciferase Assay Reagent (Promega) as recommended by the manufacturer. Total protein in the cell lysates was measured by Bio-rad Protein Assay. Relative luciferase activities are presented as percentage of activities obtained with UV-irradiated plasmids versus unirradiated control plasmids. In order to assign the XP12TA and XP25TA cells to a specific complementation group (Khan *et al*, 1998) simultaneous cotransfection was performed with 0.25 μg of an XPC cDNA containing plasmid (Legerski and Peterson, 1992), or an XPD cDNA containing plasmid (Carreau *et al*, 1995), or with pEBS7 (vector without the XPC gene; Legerski and Peterson, 1992) along with UV-irradiated or unirradiated pCMVLuc plasmid.

Figure 2. Israeli XP siblings. (*Left*) The affected proband, XP12TA, at 8 y of age. He was of Polish Jewish ancestry and had severe clinical XP with his first skin neoplasm by 2 y of age. He has marked freckling, actinic keratoses, and multiple skin cancers on sun-exposed portions of his face, arms, and neck. A skin neoplasm on his right temple was grafted with skin from a sun-shielded site resulting in a region of less pigmentation. (*Right*) The affected sister, XP25TA, at 3 y of age. She has minimal freckling on her face. She has been sun protected since birth.

RNA/DNA extraction, DNA amplification, and nucleotide sequence analysis RNA and DNA were isolated utilizing TRIzol and DNAzol reagents as per the vendor's protocol (Gibco BRL). The first strand cDNA was synthesized utilizing 2 μg total RNA as described previously (Khan *et al*, 1998) and was used to amplify the entire coding region of the XPC gene by nested polymerase chain reaction (PCR) (Li *et al*, 1993). Sequencing was performed by cycle sequencing employing dideoxy termination chemistry and an ABI 373 A Automated DNA sequencer (PE Applied Biosystems). To analyze the linkage between the exon 5 and exon 15 genetic changes, the 3.5 kb XPC cDNA was amplified, subcloned into the pCR2.1–TOPO vector (TOPO cloning kit, Invitrogen), and used in DNA sequence analysis. The mutation identified in the XPC cDNA was also confirmed by direct sequencing of the PCR amplified genomic DNA region harboring these changes.

RESULTS

Case report and pedigree **Figure 1** describes the pedigree of the investigated family [family C in Kraemer and Slor (1985)]. Both parents were clinically normal. They are descendants of Polish Ashkenazi Jewish families without known consanguinity. The family lives on a farm in Israel in a sunny area without natural shade. The family had a healthy son, 35TA, born 1971, and two affected children, a son XP12TA (the proband), born 1974, and a daughter XP25TA, born 1976. XP12TA when exposed to sunlight did not develop severe sunburn with blisters. Instead by 3 mo of age he developed markedly increased freckling on sun-exposed skin, which was later followed by actinic keratoses. His first skin cancers were basal cell carcinomas on his lip and cheek at 2 y of age. Growth and development were normal and he had no neurologic abnormalities (**Fig 2,** left). He died in 1984 at 10 y of age having more than 20 cutaneous neoplasms including four squamous cell carcinomas, two lentigo-melanomas, and multiple basal cell carcinomas involving his face, neck, and eyes. The cause of death is not known. An autopsy was not performed. The affected sister XP25TA was protected from sun exposure early in life. Her outdoor activities were limited to times when the sun was down. When going outdoors during daylight hours she used sun-protective clothing and topical sunscreens. By 1 y of age she had freckling of her face and neck. Her growth and development were normal and she had no neurologic abnormalities (**Fig 2,** right). She developed her first skin cancer at age 10 y. By age 24 y she had 10 skin neoplasms on her face, head, and neck: five basal cell carcinomas, two squamous cell carcinomas, one melanoma *in situ*, one superficial spreading melanoma, and, in 1995, an ulcerated melanoma 4.5 mm thick on her forehead.

Reduced post-UVC cell survival Cell survival was assessed by measuring post-UVC colony-forming ability of fibroblast cells obtained from XP12TA and XP25TA (affected siblings), from XPH27TA (father), from 35TA (healthy brother), and from two normal healthy donors (96TA and HSTA) (**Fig 3*A***). Parental cells and cells from the healthy brother and from the two healthy controls exhibited similar survival patterns with about 75% survival at a dose of 8 J per m^2. In marked contrast, the XP cells had about 2% survival at the same UV dose. The D_{37} (dose that results in 37% survival) was calculated to be 14 J per m^2 for the normal cells and 1.6 J per m^2 for the XP12TA and XP25TA cells, indicating that the D_{37} was 11% of normal.

Reduced UDS DNA repair measured by autoradiography showed 10% of normal post-UV UDS in XP12TA and XP25TA cells in comparison to the normal control cells C5RO and CRL1876. The XPC cell line XP1BE had 14.4% of normal post-UV UDS in the same experiment.

Reduced repair replication Post-UV nucleotide excision GGR was measured with the BrUdR-CsCl repair replication method utilizing a CsCl gradient (**Fig 3*B***). A heavy-light peak (representing non-S-phase DNA) was identified from the ratio of ^{3}H-labeled BrUdR to prelabeled cellular ^{14}C DNA. XP12TA and XP25TA showed excision repair levels of 12% and 13% compared to normal control (96TA) cells. Cells of both parents (XPH27TA and XPH26TA) exhibited DNA excision repair levels similar to the normal control 96TA cells. Cells obtained from a severe XPD patient (XP1TA) had about 8% of normal DNA repair.

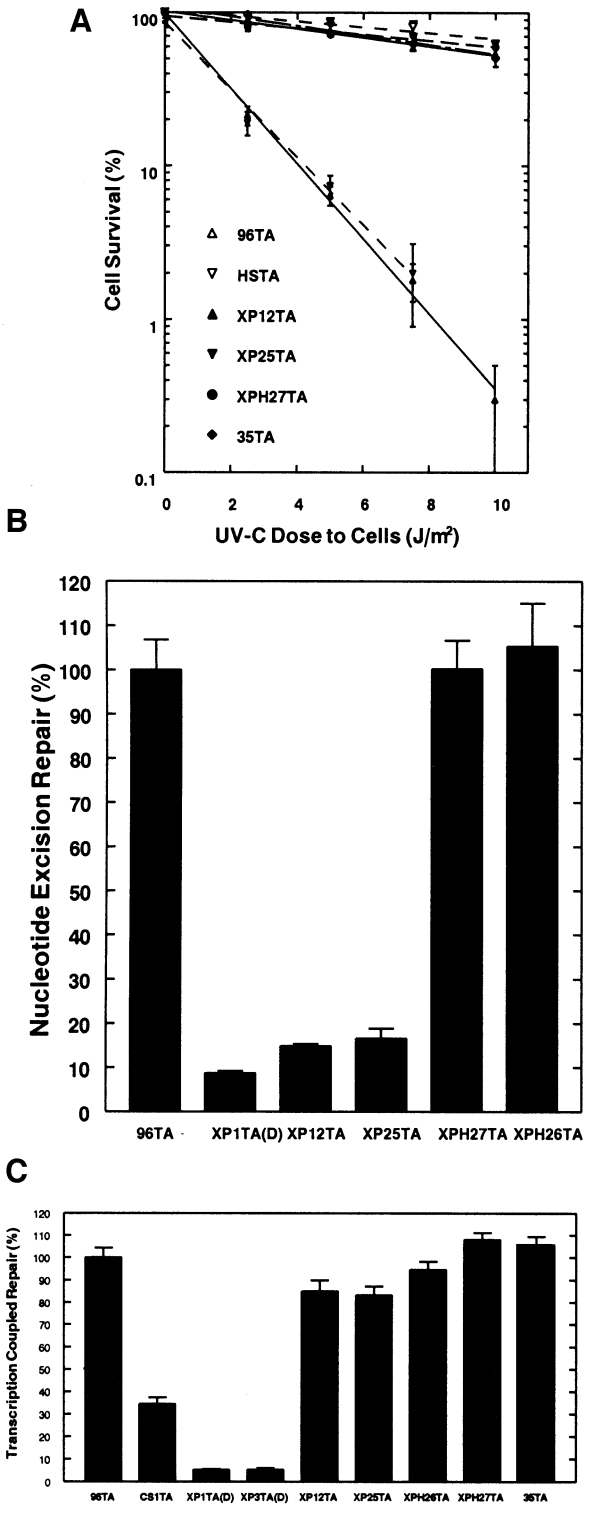
Normal TCR TCR was measured as recovery of RNA synthesis in cells 24 h after exposure to UVC irradiation (**Fig 3*C***). Fibroblasts of all members of this XP family as well as fibroblasts from the normal control (96TA), from two severe XPD cases (XP1TA and XP3TA), and from a patient with CS (CS1TA) were studied. CS cells are defective in TCR but exhibit normal GGR (Bootsma *et al*, 1998). XPC cells uniquely have normal TCR and defective GGR, whereas XPA and XPD cells have defective TCR and GGR (Bootsma *et al*, 1998). The CS cells exhibited 35% TCR, characteristic of other CS cells, and, as expected, the XPD cells exhibited less than 5% TCR (**Fig 3*C***). Cells from both parents and their normal son and cells of both XP siblings exhibited TCR in the normal range. The combination of reduced UDS with normal TCR strongly suggested that XP12TA and XP25TA belong to complementation group C.

Reduced XPC mRNA The level of XPC mRNA in cells of most, if not all, XPC patients is markedly reduced (Legerski and Peterson, 1992; Khan *et al*, 1998; Chavanne *et al*, 2000). We have measured the level of XPC mRNA in one of the affected siblings (XP25TA) and in normal cells using northern blot analysis. There was a greatly reduced level of the XPC mRNA in XP25TA cells compared to XPC mRNA in normal cells (**Fig 4,** inset). Levels of actin mRNA, used as an internal control, were similar in both cell types.

Assignment of XP cells to complementation group C Host cell reactivation of UV-irradiated luciferase reporter gene plasmid was greatly reduced in transfected XP12TA fibroblasts compared to normal cells, indicating the reduced DNA repair capacity for DNA photoproducts in these cells (**Fig 4**). Fibroblasts from both parents exhibited post-UV host cell reactivation similar to that of normal control cells (data not shown). Cotransfection of the UV-irradiated luciferase plasmid with plasmids carrying cloned XPC, XPD, or no

cDNA was performed. Only cotransfection with a plasmid containing the XPC cDNA led to a markedly increased post-UV host cell reactivation in the XPTA12 cells (**Fig 4**), thus assigning the XP12TA proband to the XPC complementation group.

DNA sequence analysis of the XPC gene Sequencing was performed on the entire 3.5 kb XPC cDNA and on selected portions of XPC genomic DNA from both patients, their parents, and the normal brother (**Fig 5**). Sequencing of the exon 5 region of genomic DNA from the clinically normal son (35TA) showed the normal DNA sequence (**Fig 5**, *top*). cDNA from the affected siblings XP12TA (**Fig 5,** *middle*) and XP25TA (data not shown) showed a homozygous deletion of two bases (AT) in exon 5 of the XPC gene at nucleotide location 669–670. We subcloned the entire 3.5 kb XPC cDNA from XP12TA. All subclones contained the del AT 669–670, supporting the observation that it was homozygous. This frameshift mutation created a new termination signal (TAA) 10 codons downstream (**Fig 5,** *middle*). Such a mutation would be expected to encode a truncated, nonfunctional 197 amino acid long XPC protein rather than the full length 940



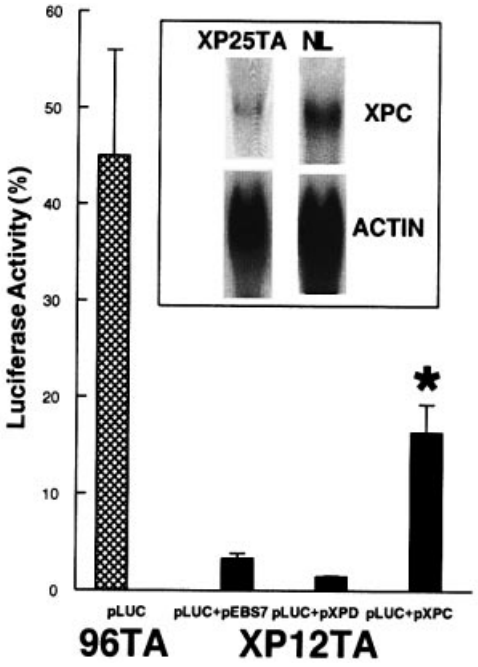


Figure 4. XPC mRNA and complementation group assignment. *Inset*: Reduced XPC mRNA in XP25TA cells. RNA was extracted from XP25TA and normal cells, separated by electrophoresis, transferred to a membrane, and probed for XPC mRNA and then for β-actin mRNA. The level of XPC mRNA was markedly reduced in XP25TA cells compared to the normal cells. Reduced post-UV host cell reactivation and assignment of XP12TA cells to XPC complementation group by host cell reactivation. XP12TA cells (*solid bars*) were transfected with 1000 J per m^2 UVC-treated plasmid pLUC along with another plasmid carrying the wild type XPD cDNA (pXPD), the wild type XPC cDNA (pXPC), or no cDNA (pEBS7). Normal cells (96TA) (*hatched bar*) were also transfected with 1000 J per m^2 UVC-treated pLUC. The data represent the mean ± SEM of triplicate samples. The XP12TA cells showed reduced plasmid host cell reactivation that was only corrected by cotransfection with the XPC cDNA containing plasmid (*) indicating that these cells are in complementation group C.

Figure 3. UV sensitivity of XP12TA and XP25TA cells. (*A*) Reduced post-UV cell survival of XP12TA and XP25TA fibroblasts. Colony-forming ability of XP12TA and XP25TA fibroblasts following exposure to various doses of UVC was reduced compared to that of the normal control fibroblasts (96TA and HSTA). Fibroblasts from the father (XPH27TA) and the unaffected brother (35TA) had normal post-UV colony-forming ability. Each point represents the mean ± SEM of triplicate samples. (*B*) Reduced nucleotide excision repair of XP12TA and XP25TA following UVC. Repair replication following exposure to 12 J per m^2 UVC was measured using ^{3}H-BrUdR labeled fibroblasts and analyzed by CsCl ultracentrifugation (details in *Materials and Methods*). The mean ± SEM of triplicate samples is presented. There was a similar reduction in nucleotide excision repair in the fibroblasts from both affected siblings (XP12TA and XP25TA). XP1TA (XPD) had reduced nucleotide excision repair in comparison to the normal cells (96TA). Fibroblasts from both parents (XPH27TA and XPH26TA) had normal excision repair. (*C*) Normal TCR of XP12TA and XP25TA following exposure to UVC. Fibroblasts were exposed to 12 J per m^2 UVC and incorporation of ^{3}H-UdR into RNA was measured immediately and then 24 h later. The recovery of RNA synthesis in triplicate samples (± SEM) is shown. CS1TA had intermediate reduction in TCR between the XPD cells (XP1TA and XP3TA) and the normal cells (96TA). The XP12TA and XP25TA cells both had nearly normal TCR.

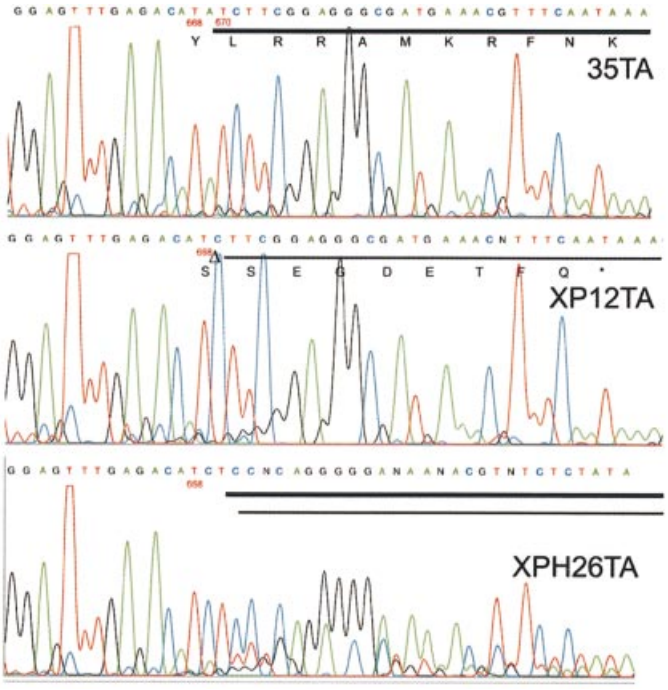


Figure 5. DNA sequence of Israeli family showing frameshift mutation in XPC exon 5. The sequence of a portion of the genomic DNA of XPC exon 5 from the normal sibling (35TA, *upper*), the affected XP patient (XP12TA, *middle*), and their mother (XPH26TA, *lower*) was determined by use of an ABI automated sequencer. The normal DNA and amino acid sequence beginning at base pair 668 is indicated by a broad horizontal line. The two-base deletion of AT at bp 669–70 (Δ) that results in a change of the amino acid sequence and a new stop codon (*) is indicated by a narrow horizontal line. 35TA has the homozygous normal sequence, XP12TA has a homozygous frameshift mutation, and XPH26TA is heterozygous for both sequences. (Note the single sequence signals in 35TA and XP12TA and double sequence signals in XPH26TA after bp 668.)

amino acid wild type XPC protein. Sequencing genomic DNA from the parents (XPH26TA, **Fig 5**, *bottom*; and XPH27TA, data not shown) indicated that both parents are heterozygous for this AT deletion in exon 5 of the XPC gene. Comparing this sequence (**Fig 5,** *bottom*) with that of the normal child (**Fig 5**, *top*) and with that of the affected child (**Fig 5**, *middle*) shows double sequences after the frameshift as one chromosome has the normal sequence and the other has a loss of two bases. Genomic DNA from XP12TA did not show this double sequence, further indicating that the deletion mutation was homozygous.

An additional transversion mutation of A to C at nucleotide 2920 was identified in exon 15. This missense mutation results in a change of amino acid 939 from lysine to glutamine. We have found that this change is a common polymorphism that retains the functional ability of the XPC cDNA to correct the defect in post-UV host cell reactivation (Khan *et al*, 2000). Subcloning of the entire 3.5 kb XPC cDNA indicates that this A2920C mutation is on the same allele that carries the AT deletion in both parents and in the affected siblings (**Fig 1**). The parents have one allele that carries both the exon 5 frameshift and the exon 15 polymorphism and another allele that has neither mutation. The affected patients are homozygous for both mutations. The unaffected brother inherited the two normal alleles from his parents.

DISCUSSION

XP clinical features and complementation groups Patients with XP are sun sensitive with early onset of freckling and skin cancer in parts of the body exposed to UV radiation (Kraemer *et al*, 1987, 1994; Bootsma *et al*, 1998; Kraemer, 1999b). The patients with classical XP have symptoms confined to sun-exposed parts of the body (skin, anterior eyes, lips, tip of the tongue). Protection from sunlight results in prevention of many of these serious abnormalities (**Fig 2**). Some XP patients, in addition, have neurologic abnormalities consisting of progressive loss of coordination, intellectual functioning, and hearing. The XP phenotype can result from a mutation in one of the seven XP genes (A-G) involved in DNA nucleotide excision repair (Bootsma *et al*, 1998), or in the XPV gene that codes for DNA polymerase η that can replicate through a damage-containing DNA template (Johnson *et al*, 1999; Masutani *et al*, 1999).

Characterization of the various mutations in XP and the resulting clinical phenotypes is essential for an understanding of the relationship between the genotype and the phenotype. Most patients with classical XP are in XP complementation groups C, E, or F but they can also be found in complementation groups A, D, and G. XP patients with neurologic abnormalities are usually in complementation groups A, B, D, or G but they can also be found in complementation group C. Thus, there is marked clinical heterogeneity in different complementation groups. Clinical heterogeneity is also found within the same complementation group. For example, a mutation in the XPD gene may cause one of four different clinical syndromes: XP without neurologic disease, XP with neurologic disease, trichothiodystrophy (a disorder with sulfur-deficient brittle hair, sun sensitivity, ichthyosis, and neurologic degeneration without cancer), or a combination of XP with CS – the XP/CS complex (Bootsma *et al*, 1998).

The XP genes are localized on different chromosomes, and the relatively few mutations identified in these genes were recently summarized (Cleaver *et al*, 1999). Only 20 mutations in 18 XP patients have been reported in the XPC gene (Li *et al*, 1993; Khan *et al*, 1998; Cleaver *et al*, 1999; Chavanne *et al*, 2000) and none was from Israel. In this investigation we have studied the genotype per phenotype relationship in two XP siblings of an Ashkenazi Jewish family.

XP in different populations XP is a very rare disease (estimated frequency 1 in 100,000 to 1 in 1,000,000); however, XP patients have been identified throughout the world in all races (Kraemer *et al*, 1987). There appears to be a higher frequency in Japan, North Africa, and parts of the Middle East. In 1985 we described 11 XP kindreds in Israel (Kraemer and Slor, 1985). Subsequently an additional 11 families have been identified. Of these 22 families, only one was Ashkenazi Jewish [family C in Kraemer and Slor (1985)].

XPC and DNA repair assays Defective nucleotide excision repair leads to increased sensitivity of cells to killing by UV radiation (**Fig 3*A***). Cells from patients in XPC have moderately increased post-UV hypersensitivity that is usually intermediate between that of XPA cells and normal cells. Heterozygotes who are carriers of XP mutations have normal post-UV cell killing.

Nucleotide excision repair can be measured by several different assays. Autoradiographic measurement of DNA repair by post-UV UDS assesses repair at the single-cell level in cells that are not in the S-phase of the cell cycle. As in our patients, UDS is typically in the range of 10%–20% of normal in XPC cells (Kraemer *et al*, 1975; Bootsma *et al*, 1998).

Repair replication, another DNA repair assay, involves growing cells in medium containing BrUdR, which is incorporated into cells in place of thymidine. BrUdR is heavier than thymidine and doubly labeled replicated strands of DNA containing BrUdR can be separated from singly labeled and unlabeled DNA by use of a CsCl gradient. Incorporation of ^{3}HTdR into singly labeled DNA after UV treatment reflects DNA repair. As most of the DNA analyzed in this assay is considered bulk, nontranscribing DNA, the assay primarily measures GGR. XP cells in complementation groups A-G are defective in GGR. XPC cells typically have reduced repair replication in the range of 10%–20% of normal (**Fig 3*B***).

DNA damage to transcribed genes is preferentially repaired in normal cells (Bootsma *et al*, 1998). XP cells in all complementation groups except XPC have defective TCR. XPC cells have normal TCR but reduced GGR. Conversely, cells from patients with CS have normal GGR and reduced TCR. We measured TCR as the recovery of RNA synthesis 24 h after exposure to UV. We found the greatest reduction in TCR in XPD cells, and an intermediate reduction in CS cells (**Fig 3*C***). The normal TCR in the cells from the XP patients (**Fig 3*C***) in combination with reduced UDS (10% of normal) and reduced GGR (**Fig 3*B***) is characteristic of XPC cells.

Low levels of mRNA are often associated with mutations that result in production of truncated proteins. This process has been called ''nonsense mediated message decay'' (Nagy and Maquat, 1998). Cells from the few XPC patients studied have low mRNA levels (Li *et al*, 1993; Khan *et al*, 1998; Chavanne *et al*, 2000) and most have truncated XPC protein. The Israeli XP patient XP25TA also has low XPC mRNA (**Fig 4**).

The ability of cells to repair DNA damage in transfected plasmids is called ''host cell reactivation''. This is an extremely sensitive assay that can detect repair of a single photoproduct in the coding sequence of the transfected gene (Protic-Sabljic and Kraemer, 1985). Reduced post-UV plasmid host cell reactivation is seen in all nucleotide excision repair defective XP cells. We found reduced post-UV plasmid host cell reactivation in XP12TA cells (**Fig 4**).

Cloning of the XP genes allowed development of a rapid complementation assay for DNA repair deficient human syndromes. Plasmids containing the cloned cDNA are cotransfected into host cells simultaneously with UV-damaged reporter plasmid (Carreau *et al*, 1995; Khan *et al*, 1998). The plasmid that corrects the DNA repair defect, as expressed in increased host cell reactivation, carries the XP gene that is defective in the host cells. Using this assay we were able to assign XP12TA to XP complementation group C (**Fig 4**).

Function of XPC protein The XP genes are involved in DNA nucleotide excision repair. The encoded proteins work in concert. They have functions such as DNA damage recognition (XPC, XPE, and XPA), unwinding the DNA by helicase activities (XPB and XPD), incisions on both sides of the lesion (XPF and XPG), re-synthesis by DNA polymerase, which uses the opposite intact strand as template, and ligation of the newly synthesized DNA to the parental DNA by DNA ligase (Lindahl and Wood, 1999). Except for XPC all other XP gene products are associated with both GGR and TCR. The XPC gene product is not involved in TCR but rather uniquely involved only in GGR and in DNA repair of the nontranscribed strands in active genes.

The XPC gene is located on chromosome 3p25.1 and encodes a 940 amino acid single stranded DNA binding protein (Bootsma *et al*, 1998). The XPC protein is tightly associated *in vivo* with another protein, HHR23B, which is encoded by a gene closely linked to the XPC gene (Masutani *et al*, 1994). The XPC-HHR23B complex acts in the initial step of damage recognition in GGR (Sugasawa *et al*, 1998), but is then released from the preincision complex before strand incision occurs (Wakasugi and Sancar, 1998). There is evidence that the XPC gene product is involved in selective repair of one type of UV photoproduct (the cyclobutane pyrimidine dimer rather than the 6–4 photoproduct) (Emmert *et al*, 2000). Transient nucleosome unfolding occurs during nucleotide excision repair, and this unfolding may require the XPC protein (Baxter and Smerdon, 1998). The XPA protein also binds to DNA damage sites following the binding by XPC-HHR23B complex where it acts as a damage verification factor (Sugasawa *et al*, 1998). As the XPC protein is not involved in TCR, it is assumed that its function is performed by a stalled RNA polymerase.

XPC mutations The biologic consequences of mutations in the XPC gene are responsible for the clinical phenotypes associated with this gene. Identification of the mutation site and type in an XPC patient is important in order to understand the correlation between a mutation and the cellular and clinical phenotypes. A mutation may alter the active site of the XPC protein, impairing its ability to bind preferentially to DNA damage sites, or its ability to bind with its associated protein HHR23B, or its ability to interact with other DNA repair factors. A mutation may create a deletion, an insertion, or an alternative splice site, which cause a frameshift that results in a premature termination codon, thus resulting in an incomplete and often short-lived mRNA and a nonsense truncated protein.

Mutations in the XPC gene have been reported in 18 other patients (Li *et al*, 1993; Khan *et al*, 1998; Cleaver *et al*, 1999; Chavanne *et al*, 2000). Most of these have been frameshift or splice mutations that lead to premature termination of the XPC message leading to a truncated protein. In this kindred XP12TA had a deletion of two bases in exon 5 leading to a premature termination 10 codons downstream. The other mutation was an A to C transversion in exon 15 leading to a change of the next to the last amino acid from lysine to glutamine. This change was present in both parents on the same allele as the deletion mutation (**Fig 1**). We have determined that this change still retains normal XPC function in an *in vivo* DNA repair assay (Khan *et al*, 2000). Further, this change was commonly seen in the XPC gene of normal individuals. Thus it is a normal polymorphism (Chavanne *et al*, 2000).

This family emigrated from Poland where they had lived for many generations. The family reported no known consanguinity. Both parents carry an allele with an AT deletion in exon 5 (del AT 669–670), however, and a functional polymorphic transversion mutation in exon 15 (A2920C) (**Fig 1**). The fact that the DNA alterations in exons 5 and 15 are identical in both parents and located on the same allele strongly suggests that they originated in a common ancestor. The healthy child has two normal XPC alleles.

CONCLUSION

This newly described frameshift mutation in the XPC gene led to reduced DNA repair with severe clinical disease, multiple skin cancers beginning at age 2 y, and death by age 10 y in one sibling. Sun protection delayed the onset of skin cancer to age 10 and prolonged life to more than 23 y in another sibling with the same mutation.

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